

Effect of colchicine on immunoregulatory abnormalities in familial Mediterranean fever

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SUMMARY

The effect of colchicine on immunoregulatory T lymphocytes in children with familial Mediterranean fever (FMF) was studied. Concanavalin A (Con A)-induced suppressor cell function was significantly ($P < 0.0001$) decreased in five untreated FMF patients ($15 \pm 3\%$, mean \pm s.e.) as compared to six age matched paediatric controls ($46 \pm 3\%$) and eight healthy adults ($49 \pm 4\%$). When the five untreated FMF patients' mononuclear cells were pre-incubated *in vitro* with Con A plus 10^{-5} M colchicine, their suppressor cell function was significantly increased ($52 \pm 10\%$, $P < 0.01$). Similarly, oral colchicine treatment (0.5 mg twice daily) significantly ($P = 0.02$) increased the five FMF patients' Con A-induced suppressor cell function to levels ($34 \pm 6\%$) that were not significantly ($P > 0.05$) different than the paediatric controls or the healthy adults. The percentage of OKT8⁺ cells (but not OKT3⁺ or OKT4⁺ cells) was significantly ($P < 0.0001$) decreased in 10 untreated FMF patients (16.0 ± 0.9) as compared to 10 paediatric controls (27.6 ± 2) or 10 healthy adults (25.7 ± 0.6). The 10 untreated FMF patients had a significant ($P < 0.002$) increase in the OKT4/OKT8 ratio (2.41 ± 0.13) as compared to 10 FMF patients treated with 0.5 mg twice daily of colchicine (1.81 ± 0.08), 10 pediatric controls (1.47 ± 0.2), or 10 healthy adults (1.78 ± 0.11). Colchicine appears to have corrected the FMF patients' elevated OKT4/OKT8 ratio by both decreasing the percentage of OKT4⁺ cells and increasing (but only partially correcting) the percentage of OKT8⁺ cells. Thus FMF patients have a suppressor cell deficiency in which colchicine treatment corrects their deficiency of Con A-induced suppressor cell function and their elevated OKT4/OKT8 ratio. This raises the possibility that colchicine might be potentially useful as an immunomodulating drug in treating patients with autoimmune or allergic diseases associated with a suppressor cell deficiency.

Keywords colchicine periodic fever T lymphocytes suppressor cells immunotherapy

INTRODUCTION

Familial Mediterranean fever (FMF) is an autosomal recessive disease characterized by recurrent attacks of fever, abdominal pain, joint pain, and/or pleuritic chest pain lasting for 1–3 days

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duration. Maintenance oral colchicine treatment prevents the acute FMF attacks of fever and polyserositis (Dinarelli *et al.*, 1974; Goldstein & Schwabe, 1974; Zemer *et al.*, 1974). We previously reported a deficiency in concanavalin A (Con A)-induced suppressor cell function in five Moslem FMF patients from one family (Ilfeld, Weil & Kuperman, 1981a) which was corrected by oral colchicine treatment (Ilfeld, Weil & Kuperman, 1981b) as well as by *in vitro* pre-incubation of the suppressor cells with Con A together with colchicine (Ilfeld & Kuperman, 1982).

In the present report, Jewish FMF patients were tested for Con A-induced suppressor cell function with and without *in vitro* colchicine as well as before and during oral colchicine treatment in order to determine whether a deficiency of Con A-induced suppressor cell function is a general phenomenon in FMF and to confirm that colchicine corrects this deficiency of Con A-induced suppressor cell function. Furthermore, the FMF patients' total T cells, helper/inducer T cells, and suppressor/cytotoxic T cells were examined with the monoclonal antibodies OKT3, OKT4 and OKT8, respectively, before and during oral colchicine treatment to determine whether the FMF patients had abnormal proportions of helper or suppressor T cells as well as to determine the effect of colchicine on helper and suppressor T cells.

MATERIALS AND METHODS

Patients. Sixteen Jewish non-Ashkenazi FMF patients (seven male and nine female) of 4–13 years of age from 11 different families were studied. All patients suffered from recurrent attacks of fever with abdominal pain and/or joint pain of 1–3 days duration. All patients had a positive family history for FMF. Blood samples were always drawn when the untreated FMF patients were between acute attacks and clinically asymptomatic (except for one patient expressly cited in the results). Ten paediatric age and sex matched patients (paediatric controls) hospitalized with mild trauma or for minor elective surgery and 10 healthy male adult volunteers (23–40 years old) served as controls. Parental informed consent was obtained prior to all venipunctures.

Surface markers for T cell subsets. One million mononuclear cells were suspended in RPMI 1640 (GIBCO, Grand Island, New York, USA) with 5% heat-inactivated fetal calf serum and mixed with 0.2 ml of monoclonal antibodies OKT3, OKT4 and OKT8 (Ortho Pharmaceutical Corp., Rariton, New York) at a final dilution of 1:40 in an ice–water bath for 30 min. After two washes with phosphate-buffered saline (PBS), the mononuclear cells were mixed with 0.1 ml of fluorescein conjugated rabbit anti-mouse IgG (Weizmann Institute, Rehovot, Israel) at a final dilution of 1:40 in an ice–water bath for 30 min. The mononuclear cells were then washed, counted and loaded onto a fluorescence activated cell sorter (FACS II, Beckton & Dickinson, Mountain View, California, USA).

Con A-induced suppressor cell function. Colchicine (Sigma Chemicals, St Louis, Missouri, USA) was dissolved in RPMI 1640 (Biolab, Jerusalem, Israel) at a concentration of 10^{-3} M, placed in test tubes wrapped with tin foil, and stored at -20°C for not more than 2 months. Five million mononuclear cells isolated on Ficoll-isopaque gradients were suspended in 1.0 ml of RPMI 1640 supplemented with 10% human heat-inactivated AB serum, penicillin, streptomycin and glutamine and put into tubes (No. 2058, Falcon, Oxnard, California) with or without $10\text{ }\mu\text{g/ml}$ of Con A (Miles Yeda, Rehovot) and with or without 10^{-5} M colchicine. Cells were incubated at 37°C in humidified 5% CO_2 –95% air mixture for 44 h, washed, and incubated for an additional 30 min with $50\text{ }\mu\text{g/ml}$ mitomycin C (Sigma Chemicals, Ohio, USA). After four washings with PBS, these suppressor cells (5×10^4) were co-cultured with 5×10^4 fresh allogeneic responder cells from healthy volunteers in microplates (No. 4506, Costar, Cambridge, Massachusetts, USA) in 0.1 ml culture medium with or without phytohemagglutinin (PHA, Burroughs Wellcome, England). After 68 h of incubation, tritiated thymidine was added for an additional 4 h. Cells were harvested and the radioactivity measured in a Packard liquid scintillation counter. Suppressor cell function was calculated according to the following formula:

$$\% \text{ suppression} = 1 - \frac{\text{ct/min (RC + CSC + PHA)} - \text{ct/min (RC + CSC)}}{\text{ct/min (RC + MSC + PHA)} - \text{ct/min (RC + MSC)}} \times 100\%$$

where RC represents responder cells, MSC represents suppressor cells incubated in medium, and CSC represents suppressor cells incubated with Con A and/or colchicine.

Statistics. Results are presented as mean \pm s.e. and significance was computed by the Student's *t*-test.

RESULTS

Five untreated FMF patients had a significant ($P < 0.0001$) decrease in Con A-induced suppressor cell function as compared to six paediatric controls and eight healthy adults (Tables 1 & 2). Oral colchicine treatment (0.5 mg twice daily) significantly ($P = 0.02$) increased the FMF patients' Con

Table 1. Effect of *in vitro* colchicine on Con A-induced suppressor cell function

Donor	Pre-incubation of suppressor cells	Proliferation (ct/min)		% Suppression
		Medium	PHA	
Normal	Medium	605	42,581	0
	Colchicine	703	54,644	-28
	Con A	1,028	25,120	43
	Con A + Colchicine	1,156	53,216	-24
FMF	Medium	1,341	59,343	0
	Colchicine	1,951	63,585	-6
	Con A	2,311	49,913	18
	Con A + Colchicine	2,991	11,170	86

Suppressor cells from one healthy adult and one untreated FMF patient were pre-incubated with medium, 10^{-5} M colchicine, Con A or 10^{-5} M colchicine together with Con A. After 44 h, these suppressor cells were washed, pulsed with mitomycin-C, washed, and then co-cultured with medium or PHA plus responder cells from healthy volunteers for 72 h. Percentage suppression of proliferation was calculated as the percentage difference between the net ct/min of tritiated thymidine uptake (PHA stimulated minus medium stimulated) of suppressor cells pre-incubated with 10^{-5} M colchicine and/or Con A as compared to suppressor cells pre-incubated with medium.

Table 2. Effect of *in vitro* and *in vivo* colchicine on Con A-induced suppressor cell function

Donor of suppressor cells	Pre-incubation of suppressor cells		
	Colchicine	Con A	Con A + Colchicine
FMF untreated ($n = 5$)	16 \pm 9	15 \pm 3	52 \pm 10
FMF colchicine 1 mg/day ($n = 5$)	N.T.	34 \pm 6	N.T.
Paediatric controls ($n = 6$)	-2 \pm 6	46 \pm 3	-8 \pm 7
Healthy adults ($n = 8$)	11 \pm 3	49 \pm 4	-22 \pm 10

Suppressor cells from 5 untreated FMF patients, five FMF patients treated with oral colchicine (0.5 mg twice daily), six paediatric controls, and eight healthy adults were pre-incubated for 44 h with medium, 10^{-5} M colchicine and/or Con A. The data represent the mean (\pm s.e.) percentage suppression of proliferation by suppressor cells pre-incubated with colchicine and/or Con A as compared to suppressor cells pre-incubated with medium. N.T. = Not tested.

A-induced suppressor cell function to levels that were not significantly ($P > 0.05$) different from the paediatric controls or the healthy adults. Similarly, pre-incubation of the untreated FMF patients' suppressor cells with Con A plus 10^{-5} M colchicine significantly ($P < 0.01$) increased their suppressor cell function. In contrast, pre-incubation of the pediatric controls' and healthy adults' suppressor cells with Con A plus 10^{-5} M colchicine significantly ($P < 0.0001$) decreased their suppressor cell function. There was no significant difference in controls' or FMF patients' cell yield or cell viability (ascertained by exclusion of trypan blue) among suppressor cells pre-incubated for 44 h in medium, 10^{-5} M colchicine, Con A, or Con A together with 10^{-5} M colchicine (data not shown).

Ten healthy adults, 10 paediatric controls, 10 untreated FMF patients and 10 FMF patients treated with 0.5 mg twice daily of colchicine were tested with monoclonal antibodies (Table 3 & Fig. 1). The untreated FMF patients had similar percentage of OKT3⁺ cells and OKT4⁺ cells as the

Table 3. Effect of oral colchicine treatment of FMF patients on subpopulations of T lymphocytes as measured by monoclonal antibodies

Donor of mononuclear cells	%OKT3 ⁺	%OKT4 ⁺	%OKT8 ⁺	OKT4/OKT8
FMF untreated ($n = 10$)	66.1 ± 3.0	37.1 ± 0.9	16.0 ± 0.9	2.41 ± 0.13
FMF colchicine 1 mg/day ($n = 10$)	60.3 ± 1.7	35.1 ± 1.1	19.4 ± 0.7	1.81 ± 0.08
Paediatric controls ($n = 10$)	65.4 ± 4.6	38.0 ± 3.9	27.6 ± 2.0	1.47 ± 0.20
Healthy adults ($n = 10$)	75.5 ± 1.8	45.2 ± 2.0	25.7 ± 0.6	1.78 ± 0.11

The data represent the mean (±s.e.) percentage of mononuclear cells positively staining with OKT3, OKT4 or OKT8.

paediatric (but not adult) controls. In contrast, the untreated FMF patients had significantly ($P < 0.0001$) decreased percentage of OKT8⁺ cells and a significantly ($P < 0.001$) increased ratio of OKT4/OKT8 as compared to the paediatric or adult controls. There was no significant difference between the ratio of OKT4/OKT8 in the six untreated female FMF patients (2.49 ± 0.2) as compared to the four untreated male FMF patients (2.27 ± 0.11). Comparing the 10 untreated FMF patients with the 10 treated FMF patients, there was a mild but not significant decrease of the percentage of OKT3⁺ cells and OKT4⁺ cells during colchicine treatment ($P = 0.11$ and $P = 0.18$, respectively); whereas there was a significant ($P = 0.01$) increase in the percentage of OKT8⁺ cells and a significant ($P = 0.001$) decrease in the ratio of OKT4/OKT8 during colchicine treatment (Table 3). When only the six FMF patients who were tested both before and during colchicine treatment were compared (using a paired *t*-test), there was a mild but not significant ($P = 0.14$) increase of the percentage of OKT8⁺ cells, a significant ($P = 0.01$) decrease of both the percentage of OKT3⁺ cells and OKT4⁺ cells, and a significant ($P = 0.003$) decrease of the ratio of OKT4/OKT8 (Fig. 1). There was no significant difference in the ratio of OKT4/OKT8 comparing the 10 colchicine treated FMF patients with either the 10 healthy adults or the 10 paediatric controls ($P = 0.83$ and $P = 0.13$, respectively). In contrast, colchicine treated FMF patients still had a significant ($P < 0.0001$) decrease in the percentage of OKT8⁺ cells as compared to the paediatric controls or healthy adults. Thus oral colchicine treatment appears to have corrected the elevated OKT4/OKT8 ratio in the FMF patients by decreasing the percentage of OKT4⁺ cells and increasing (but only partially correcting) the percentage of OKT8⁺ cells. Six healthy untreated adults and nine adult untreated control patients were tested twice each and there was no significant difference in the ratio of OKT4/OKT8 comparing each person's first and second tests (data not shown). Therefore, the decrease in the FMF patients' ratio of OKT4/OKT8 is due to the oral colchicine treatment rather than any technical changes such as from the antibodies or the fluorescence activated cell sorter.

Three FMF patients had an elevated OKT4/OKT8 ratio which was decreased after 1 week of oral colchicine treatment (data not shown). One patient was tested during an acute attack of FMF.

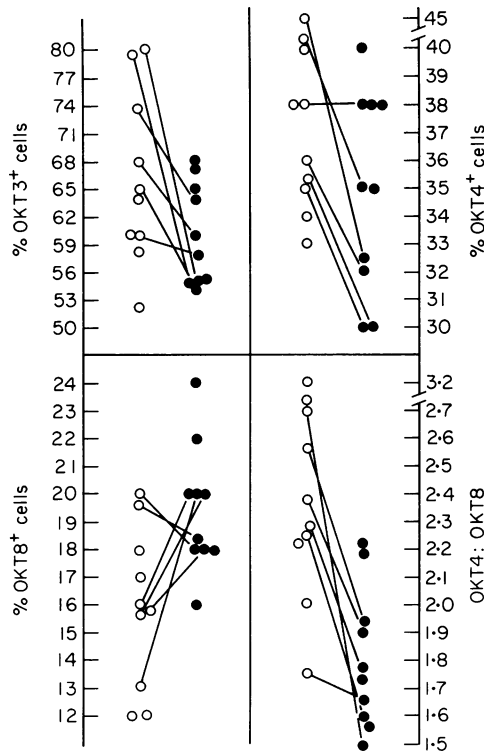


Fig. 1. Measurement of T lymphocyte subsets with monoclonal antibodies in 10 untreated FMF patients (○) and 10 FMF patients receiving 0.5 mg twice daily of oral colchicine treatment (●). A line connects the proportions of T lymphocytes before and during colchicine treatment for the six FMF patients tested both before and during oral colchicine treatment.

His ratio of OKT4/OKT8 was 2.25 and his Con A-induced suppressor cell function was -1% without *in vitro* colchicine and 57% with *in vitro* colchicine. After 1 week of oral colchicine, 0.5 mg twice daily, his Con A-induced suppressor cell function was 51% (without *in vitro* colchicine) even though he later had a few FMF attacks. These findings confirm and extend the previous observation (Ilfeld & Kuperman, 1982) that oral colchicine treatment corrects the deficiency of Con-A induced suppressor cell function within 1 week of starting treatment regardless of whether or not FMF attacks are totally prevented.

We examined the possibility of an aberration in the percentage of OKT3⁺, OKT4⁻, OKT8⁻ cells (assuming no cells stained for both OKT4 and OKT8) by subtracting the percentage of OKT4⁺ cells plus OKT8⁺ cells from the percentage of OKT3⁺ cells. The percentage of OKT3⁺, OKT4⁻, OKT8⁻ cells was significantly ($P < 0.05$) increased in the 10 untreated FMF patients (12.6 ± 3.0) as compared to either the 10 colchicine treated FMF patients (6.1 ± 0.8), the 10 paediatric controls (-2.9 ± 5.8), or the 10 healthy adults (4.6 ± 1.3). It is not known whether this method of calculation is appropriate but regarding biological significance it does suggest that FMF patients have an abnormal appearance of OKT3⁺, OKT4⁻, OKT8⁻ cells and that colchicine treatment may have modulated three subpopulations of T lymphocytes in the FMF patients. There is no significant overlap between OKT4⁺ cells and OKT8⁺ cells in healthy volunteers although significant numbers of double labelled OKT4⁺, OKT8⁺ cells have been reported in a few diseases such as severe combined immunodeficiency syndromes, myasthenia gravis and chronic active hepatitis (Bach & Bach, 1981). We did not examine the possibility of double labelled OKT4⁺, OKT8⁺ cells in the FMF patients but this could not explain the abnormal increase of the percent of OKT3⁺, OKT4⁻, OKT8⁻ cells since double labelled OKT4⁺, OKT8⁺ cells should have decreased (rather than increased) the calculation of OKT3⁺, OKT4⁻, OKT8⁻ cells.

DISCUSSION

These results confirm that untreated FMF patients have a deficiency of Con A-induced suppressor cell function which can be corrected by either oral colchicine treatment or *in vitro* pre-incubation with colchicine. Con A can activate helper T cells as well as suppressor T cells so the deficiency of Con A-induced suppressor cell function in untreated FMF patients may be due to increased helper T cell function and/or decreased suppressor T cell function. The observation that untreated FMF patients have a normal percentage of OKT4⁺ helper/inducer T cells and a decreased percentage of OKT8⁺ suppressor/cytotoxic T cells suggests that the deficiency of Con A-induced suppressor cell function in untreated FMF patients is probably due to decreased suppressor T cell function rather than excessive helper T cell function. Oral colchicine treatment appeared to decrease the proportion of OKT4⁺ cells and to increase (but only partially correct) the proportion of OKT8⁺ cells; however, further experiments are needed to ascertain whether colchicine modulates OKT4⁺ helper cells as well as OKT8⁺ suppressor cells. For example, oral colchicine treatment may have decreased helper T cell numbers and/or function as well as increased suppressor T cell numbers and/or function; however, a definite conclusion cannot be reached because a subset of OKT4⁺ cells mediates suppression in functional assays (Thomas *et al.*, 1981; Morimoto *et al.*, 1983) and OKT8⁺ cells include cytotoxic cells as well as suppressor cells. Furthermore, oral colchicine treatment may also have modulated Con A-induced suppressor cell function via monocytes. This observation of oral colchicine treatment modulating at least two subpopulations of immunoregulatory T cells as determined with monoclonal antibodies is consistent with the previous observation of oral colchicine treatment modulating two different lymphocyte functions in the Con A-induced suppressor cell assay (Ilfeld, Weil & Kuperman, 1982).

It is interesting to compare our results with the study of Nouri-Aria *et al.* (1982) that patients with autoimmune chronic active hepatitis have a severe deficiency of Con A-induced suppressor cell function which is corrected by *in vitro* pre-incubation with prednisolone as well as oral prednisolone treatment. In contrast, patients with hepatitis B surface antigen (HBsAg) positive chronic active hepatitis only have a mild deficiency of Con A-induced suppressor cell function which was mildly decreased by *in vitro* pre-incubation with prednisolone and unaffected by oral prednisolone treatment (Nouri-Aria *et al.*, 1982). There is a similarity between their findings and our observations in that *in vitro* pre-incubation as well as oral treatment with a drug (prednisolone or colchicine) significantly increased Con A-induced suppressor cell function for patients (autoimmune chronic active hepatitis or FMF) with a severe suppressor cell deficiency whereas *in vitro* pre-incubation with the same drug (prednisolone or colchicine) mildly or markedly decreased Con A-induced suppressor cell function in those (HBsAg positive chronic active hepatitis or healthy volunteers) with mildly decreased or normal suppressor cell function (respectively).

The suppressor cell deficiency in FMF may be due to non-specific depression by disease activity or it may be a genetic marker for FMF or part of the pathogenesis of FMF. The suppressor cell deficiency is probably not due to non-specific depression by disease activity since all but one of the untreated FMF patients were tested in between attacks when they were clinically asymptomatic. The suppressor cell deficiency is probably not a genetic marker since FMF patients' healthy family members who should have been heterozygous for this autosomal recessive disease did not have a suppressor cell deficiency (Ilfeld *et al.*, 1981a). Therefore, the most likely explanation is that the suppressor cell deficiency in FMF may be associated with the pathogenesis of the disease. This raises the possibility that oral colchicine treatment may, in part, prevent FMF attacks by modulating immunoregulatory cells.

An important question is whether colchicine directly corrected the suppressor cell deficiency in FMF and thus might be useful as an immunomodulating drug in other diseases associated with a suppressor cell deficiency or whether colchicine prevented FMF attacks by unknown mechanisms which only secondarily resulted in the correction of the suppressor cell deficiency. The following four observations suggest that colchicine directly modulated immunoregulatory cells. First, *in vitro* colchicine corrected FMF patients' Con A-induced suppressor cell function. Second, the correction of the deficiency of Con A-induced suppressor cell function and the elevated OKT4/OKT8 ratio

within 1 week of starting oral colchicine treatment could not be via prevention of FMF attacks because FMF attacks usually do not occur within 7 days. Third, Con A-induced suppressor cell function was corrected by oral colchicine treatment even though two patients still had some FMF attacks. Fourth, preliminary studies of two adult FMF patients who had no FMF attacks on either 1 or 2 mg/day of colchicine suggested a dose-response between colchicine and Con A-induced suppressor cell function (Ilfeld & Kuperman, 1982). Thus oral colchicine treatment appears to directly modulate FMF patients' immunoregulatory cells raising the possibility that colchicine might have potential therapeutic use as an immunomodulating drug in treating patients with autoimmune or allergic diseases associated with a suppressor cell deficiency.

Oral colchicine treatment has been reported to be clinically useful in treating patients with cirrhosis (Kershenovich *et al.* 1979), psoriasis (Wahba & Cohen, 1980) and Behçet's disease (Matsumura & Mizushima, 1975; Aktulga *et al.*, 1980). Interestingly, patients with cirrhosis (Hodgson, Wands & Isselbacher, 1978; Kawanishi *et al.*, 1981), psoriasis (Sauder *et al.*, 1980) and Behçet's disease (Sakane *et al.*, 1982) have been reported to have a deficiency of Con A-induced suppressor cell function. Further investigations are needed to study the clinical and immunoregulatory effects of oral colchicine treatment in patients with these diseases as well as with other autoimmune or allergic diseases associated with a suppressor cell deficiency.

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